and Moore (1943). According to Price (1939) there are no specimens of *S. polyorchis* available and except for differences in number of testes (20–23 in *S. polyorchis* and 12–16 in *S. osleri*) and in the supposed absence of spines on large haptoral hooks of *S. polyorchis*, the 2 species are essentially the same in all other characteristics and measurements. Compelling evidence for recognizing distinct species is not evident and it is possible that the differences noted by Alvey (1936) may be due to individual variation. Therefore, the synonymy originally proposed by Price (1939) is provisionally supported until specimens of *S. polyorchis* can be rediscovered and examined.

S.E.T. thanks the Arkansas Game and Fish Commission for Scientific Collecting Permit No. 831.

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J. Helminthol. Soc. Wash.
58(1), 1991, pp. 140–142

Research Note

Long-term Storage of Hookworm Infective Larvae in Buffered Saline Solution Maintains Larval Responsiveness to Host Signals

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ABSTRACT: Third-stage larvae (L_3's) of *Ancylostoma caninum* stored in water exhibited a decline in the number of larvae that resumed feeding in response to canine serum, whereas those stored in copro-culture for the same amount of time failed to show this decline. When L_3's were stored for 39 days in BU, a *Caenorhabditis elegans* handling buffer, they retained the ability to resume feeding. Short (<24 hr) storage in water had no effect on feeding.

KEY WORDS: *Ancylostoma caninum*, host signals, hookworm, infective larvae.

During investigations of the resumption of feeding by infective hookworm larvae (L_3's) when exposed to host-mimicking conditions in vitro (Hawdon and Schad, 1990), we have observed a marked decrease in the proportion of larvae responding to a feeding stimulus when the larvae were first stored in water. Larvae that remained in copro-culture for the same length of time failed to exhibit this decline in the proportion feeding,

Table 1. Effect of storage conditions on the feeding of third-stage larvae of the hookworm *Ancylostoma caninum*.

Storage conditions	Mean % feeding ± SD*
None	61.9 ± 8.9[a]
39 days in culture	70.1 ± 4.1[a]
39 days in dH_2O	23.3 ± 0.6[b]
39 days in BU†	68.0 ± 2.6[a]

* Mean of 3 replicates; SD = standard deviation. Values with different superscripts are significantly different at $P < 0.05$ using Student's *t*-test on arcsin transformed data.

† BU = 50 mM Na_2HPO_4/22 mM KH_2PO_4/70 mM NaCl. See text for details.

Table 2. Effect of axenization in distilled water and BU on the resumption of feeding by third-stage larvae of *Ancylostoma caninum* in vitro.

Axenization medium	Serum	Mean % feeding ± SD*
dH_2O	+	60.4 ± 4.1[a]
	−	3.3 ± 0.6[b]
BU	+	61.4 ± 4.1[a]
	−	1.7 ± 1.6[b]

* SD = standard deviation; $N = 3$. Values with different superscripts are significantly different at $P < 0.05$.

suggesting that conditions in culture were more favorable for maintaining responsiveness to the host signals encountered during infection. One possible explanation for these observations was the difference in solute concentration in the storage media. Therefore, an experiment was designed to test this hypothesis.

Ancylostoma caninum L_3's were recovered from copro-cultures by a Baermann technique, and washed 3 times in sterile distilled water (dH_2O). The pellet was suspended in 20 ml of sterile dH_2O containing penicillin (100 U/ml), streptomycin (100 μg/ml), and tetracycline (1 mg/ml) for axenization. The worms were transferred to sterile glass petri dishes and incubated overnight at 25°C. After axenization, the larvae were counted and divided into 3 equal groups. One group was washed 3 times with sterile dH_2O, while a second was washed 3 times with sterile BU, a buffer used in the handling of the free-living nematode, *Caenorhabditis elegans* (50 mM Na_2HPO_4/22 mM KH_2PO_4/70 mM NaCl, pH 6.8; Clokey and Jacobson, 1986). Antibiotics were not included in the wash solutions. The final pellets were suspended in 20 ml of the appropriate solution supplemented with penicillin (100 U/ml), streptomycin (100 μg/ml), and amphotericin B (5 μg/ml), and transferred to sterile glass petri plates. The plates were incubated for 39 days at 25°C, with a light : dark cycle of 11:13. To establish a baseline feeding activity, the third group was incubated under host-like conditions immediately after axenization and assayed for feeding 24 hr later as described previously (Hawdon and Schad, 1990). After the 39-day incubation period, the dH_2O- and BU-groups were also incubated and assayed for feeding as above. Larvae harvested from 39-day-old copro-cultures (i.e., cultured from the same fecal sample) served as controls. The results are shown in Table 1. The proportion of larvae stored in BU that were feeding did not differ ($P > 0.05$) from that observed among larvae assayed immediately (i.e., no treatment), or those recovered directly from 39-day copro-cultures. In sharp contrast, a markedly smaller proportion of larvae fed after storage in dH_2O. There also appeared to be higher mortality in water-stored larvae, although this was not evaluated quantitatively.

To determine if even the short axenization in water adversely affected feeding, larvae from a single batch of copro-cultures were either axenized in dH_2O or BU for 14 hr and then assayed for feeding. There was no significant difference ($P > 0.05$) in the proportion of larvae feeding (Table 2), indicating that processing larvae in dH_2O for a short period (<24 hr) had no effect on their ability to resume feeding.

These results suggest that storage in the buffer BU instead of distilled water may maintain the infectivity of hookworm larvae stored for extended periods of time. Third-stage larvae of hookworms lose infectivity with storage, generally thought to be the result of decreased metabolic rates and lipid reserves associated with aging (Clark, 1969; Croll and Matthews, 1973). Storage in near-isotonic solutions may conserve energy by decreasing the activity of the excretory ampulla, a structure used in maintaining water balance (Weinstein, 1952; Croll et al., 1972). However, Croll (1972) suggests that maintenance of water balance requires minimal energy expenditure, and that lipid use in *Ancylostoma tubaeforme* L_3's is approaching maximum at NaCl concentrations equivalent to that of BU. Alternatively, storage in distilled water may induce a quiescent or moribund state in which larvae are unable to respond to host signals upon infection.

Conversely, storage in BU may keep the larvae within their "activity sphere" (Croll, 1972), i.e., the optimum physiological parameters for activity and survival, thereby allowing them to remain responsive to environmental signals. Indeed, larvae of *A. ceylanicum* and *Necator americanus* stored in BU for 3–4 wk were capable of causing patent infections in hamsters, although their infectivity compared to water-stored larvae was not examined. The percentage of hookworm L_3's that resume feeding in vitro has not been correlated directly with infectivity, but the level of feeding exhibited by larvae stored in BU suggests that these larvae retain the ability to respond to the host signals encountered during infection.

We thank Lan Han for expert technical assistance in maintaining the parasite. This work was supported in part by NIH Grants 5RO1-AI-22662 and BRSG 507RRB5464.

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J. Helminthol. Soc. Wash.
58(1), 1991, pp. 142–146

Research Note

Helminths of Three Toads, *Bufo alvarius, Bufo cognatus* (Bufonidae), and *Scaphiopus couchii* (Pelobatidae), from Southern Arizona

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ABSTRACT: The gastrointestinal tracts and lungs of 3 toad species were examined for helminths. Examination of 95 *Bufo alvarius* revealed the presence of the nematodes *Aplectana itzocanensis* Bravo Hollis, 1943, *Physaloptera* sp. Rudolphi, 1819, *Physocephalus* sp. Diesing, 1861, *Oswaldocruzia pipiens* Walton, 1929, the cestode *Nematotaenia dispar* (Goeze, 1782) Lühe, 1899, in the gastrointestinal tract, and the nematode *Rhabdias americanus* Baker, 1978, in the lungs. *Bufo cognatus* ($N = 21$) had the nematodes *A. itzocanensis, O. pipiens, Physaloptera* sp., and the cestode *Distoichometra bufonis* Dickey, 1921, in the gastrointestinal tract. The nematode *R. americanus* was found in the lungs. *Scaphiopus couchii* ($N = 76$) had the nematodes *Aplectana incerta* Caballero, 1949, and *O. pipiens,* and the cestode *D. bufonis* in the digestive trace. No helminths were found in the lungs of *S. couchii.*

KEY WORDS: Nematoda, *Aplectana incerta, Aplectana itzocanensis, Physaloptera* sp., *Physocephalus* sp., *Oswaldocruzia pipiens, Rhabdias americanus,* Cestoda, *Distoichometra bufonis, Nematotaenia dispar,* prevalence, intensity, survey, Bufonidae, *Bufo alvarius, Bufo cognatus,* Pelobatidae, *Scaphiopus couchii.*

The Colorado River toad, *Bufo alvarius,* the Great Plains toad, *Bufo cognatus,* and Couch's spadefoot, *Scaphiopus couchii,* occur sympatrically in southern Arizona. *Bufo alvarius* ranges across southern Arizona and extreme southwestern New Mexico to northwest Sinaloa, Mexico from sea level to 1,610 m. *Bufo cognatus* has a geographic range extending from extreme